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(54) Title: DESIGN OF HIGH AFFINITY RNASE H RECRUITING OLIGONUCLEOTIDE

(57) Abstract: The present invention relates to the field of bicyclic DNA analogues, e.g. LNA and LNA modifications, which are useful for designing oligomers that form high affinity duplexes with complementary RNA wherein said duplexes are substrates for RNase H. The oligonucleotides may be partially or fully composed of LNA analogues with very high affinity and ability to recruit RNase H. The implications are that oxy-LNA by itself may be used to construct novel antisense molecules with enhanced biological activity. Alternatively, oxy-LNA may be used in combination with non-oxy-LNA, such as standard DNA, RNA or other analogues, e.g. thio-LNA or amino-LNA, to create high affinity, RNase H recruiting anti-sense compounds without the need to adhere to any fixed design.

Design of high affinity RNase H recruiting oligonucleotide

Field of Invention

The present invention relates to the field of bicyclic DNA analogues which are useful for designing oligomers that forms high affinity duplexes with complementary RNA wherein said duplexes are substrates for RNase H. The oligonucleotides may be partially or fully composed of bicyclic DNA analogues.

Background of the invention

The term "antisense" relates to the use of oligonucleotides as therapeutic agents. Briefly, an antisense drug operates by binding to the mRNA thereby blocking or modulating its translation into protein. Thus, antisense drugs may be used to directly block the synthesis of disease causing proteins. It may, of course, equally well be used to block synthesis of normal proteins in cases where these participate in, and aggravate a pathophysiological process. Also, it ought to be emphasised that antisense drugs can be used to activate genes rather that suppressing them. As an example, this can be achieved by blocking the synthesis of a natural suppressor protein.

Mechanistically, the hybridising oligonucleotide is thought to elicit its effect by either creating a physical block to the translation process or by recruiting a cellular enzyme (RNase H) that specifically degrades the mRNA part of the mRNA/antisense oligonucleotide duplex.

Not unexpectedly, oligonucleotides must satisfy a large number of different requirements to be useful as antisense drugs. Importantly, the antisense oligonucleotide must bind with high affinity and specificity to its target mRNA, must have the ability to recruit RNase H, must be able to reach its site of action within the cell, must be stable to extra – and intracellular nucleases both endo- and exo-nucleases, must be non-toxic/minimally immune stimulatory, etc.

Natural DNA only exhibit modest affinity for RNA and fall short on a number of the other critical characteristics, especially nuclease resistance. Hence, a significant effort has been invested to identify novel analogues with improved antisense properties. In particular the search has focused on identifying novel analogues, which combine an increased affinity

for complementary nucleic acids with the RNase H recruiting ability of natural DNA. Both of these properties have been demonstrated to correlate in a strongly positive manner with biological activity. Of the vast number of analogues that have emerged from this work, only few retain the ability to recruit RNase H and very few provide useful increases in affinity. Sadly, those that do provide a useful increase in affinity fail to recruit RNase H.

In the face of these results the field have turned to mixed backbone oligonucleotides as a means to provide higher potency antisense drugs, *i.e.* antisense molecules that operates by a two fold mechanism of action 1) high affinity mediated translational arrest at the ribosomal level and 2) activation of RNase H. These molecules (termed gab-mers) typically comprise a central region of at least six contiguous, low affinity phosphorothioates (RNase H recruiting analogues) flanked by stretches of high affinity analogues (non RNase H recruiting analogues) that enhance the ability to promote translational arrest. Although expected to out-perform current phosphorothioate antisense drugs, the gab-mers are not considered the ideal antisense molecules. Amongst their weaknesses is the requirement for a rather fixed design and the presence of high and low affinity domains within the molecule, which may compromise biological activity.

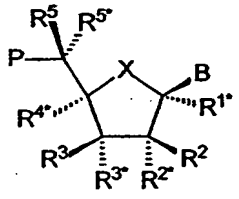
The enzyme RNase H selectively binds to heterogeneous DNA/RNA duplexes and degrades the RNA part of the duplex. Homogeneous DNA/DNA and RNA/RNA duplexes, which only differs molecularly from the DNA/RNA duplex at the 2′ position (**DNA/DNA:** 2′-H/2′-H; **RNA/RNA:** 2′-OH/2′-OH and **DNA/RNA:** 2′-H/2′-OH) are not substrates for the enzyme. This suggests that either the molecular composition at the 2′ position itself or the structural feature it imposes on the helix is vital for enzyme recognition. Consistent with this notion, all 2′-modified analogues that have so far been reported to exhibit increased affinity have lost the ability to recruit RNase H.

Detailed description of the invention.

Locked Nucleic Acid (LNA) is a novel, nucleic bicyclic acid analogue in which the 2′- and 4′ position of the furanose ring are linked by an O-methylene (oxy-LNA), S-methylene (thio-LNA) or NH_2-methylene moiety (amino-LNA). This linkage restricts the conformational freedom of the furanose ring and leads to an increase in affinity which is by far the highest ever reported for a DNA analogue (WO 99/14226).

Despite the fact that the modification in LNA involves the 2'-position we have found that the activity of RNase H is not dependent on a contiguous stretch of DNA or phosphorothioated bases when oxy-LNA is used as a component of the oligonucleotide. In fact, we have found that oligonucleotides composed entirely of oxy-LNA are able to recruit RNase H. Oxy-LNA oligonucleotides thus constitutes the first ever DNA analogue to display the long sought after combination of very high affinity and ability to recruit RNase H. The implications are that oxy-LNA by itself may be used to construct novel antisense molecules with enhanced biological activity. Alternatively, oxy-LNA may be used in combination with non-oxy-LNA, such as standard DNA, RNA or other analogues, e.g. thio-LNA or amino-LNA to create high affinity, RNase H recruiting antisense compounds without the need to adhere to any fixed design.

An "oxy-LNA monomer" is defined herein as a nucleotide monomer of the formula Ia



Ia

wherein X is oxygen; B is a nucleobase; R^{1*}, R^2, R^3, R^5 and R^{5*} are hydrogen; P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, R^{3*} is an internucleoside linkage to a preceding monomer, or a 3'-terminal group; and R^{2*} and R^{4*} together designate -O-CH_2- where the oxygen is attached in the 2'-position.

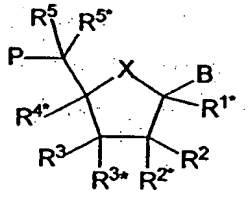
The term "nucleobase" covers the naturally occurring nucleobases adenine (A), guanine (G), cytosine (C), thymine (T) and uracil (U) as well as non-naturally occuring nucleobases such as xanthine, diaminopurine, 8-oxo-N^6-methyladenine, 7-deazaxanthine, 7-deazaguanine, N^4,N^4-ethanocytosin, N^6,N^6-ethano-2,6-diaminopurine, 5-methylcytosine, 5-(C^3-C^6)-alkynylcytosine, 5-fluorouracil, 5-bromouracil, pseudoisocytosine, 2-hydroxy-5-methyl-4-triazolopyridin, isocytosine, isoguanin, inosine and the "non-naturally occurring" nucleobases described in Benner et al., U.S. Pat No. 5,432,272 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol. 25, pp 4429-4443. The term "nucleobase" thus includes not only the known purine and pyrimidine heterocycles, but also heterocyclic analogues and tautomers thereof. It should be clear to the person skilled in

the art that various nucleobases which previously have been considered "non-naturally occurring" have subsequently been found in nature.

A "non-oxy-LNA" monomer is broadly defined as any nucleoside (i.e. a glycoside of a heterocyclic base) which is not itself an oxy-LNA but which can be used in combination with oxy-LNA monomers to construct oligos which have the ability to bind sequence specifically to complementary nucleic acids. Examples of non-oxy-LNA monomers include 2'-deoxynucleotides (DNA) or nucleotides (RNA) or any analogues of these monomers which are not oxy-LNA, such as for example the thio-LNA and amino-LNA described by Wengel and coworkers (Singh et al. J. Org. Chem. 1998, 6, 6078-9, and the derivatives described in Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443.

It should be understood that the incorporation of non-oxy-LNA monomer(s) into an oxy-LNA oligo may change the RNAseH recruiting characteristics of the oxy-LNA/non-oxy-LNA chimeric oligo. Thus, depending on the number and type of non-oxy-LNA monomer(s) used, and the position of these monomers in the resulting oxy-LNA/non-oxy-LNA chimeric oligo, the chimera may have an increased, unaltered or decreased ability to recruit RNAsdeH as compared to the corresponding all oxy-LNA oligo.

As mentioned above, a wide variety of modifications of the deoxynucleotide skeleton can be contemplated and one large group of possible non-oxy-LNA can be described by the following formula I



I

wherein X is -O-; B is selected from nucleobases; R^{1*} is hydrogen;

P designates the radical position for an internucleoside linkage to a succeeding monomer, or a 5'-terminal group, such internucleoside linkage or 5'-terminal group optionally including the substituent R^5, R^5 being hydrogen or included in an internucleoside linkage,

R^{3*} is a group P* which designates an internucleoside linkage to a preceding monomer, or a 3'-terminal group;

one or two pairs of non-geminal substituents selected from the present substituents of R^2, R^{2*}, R^3, R^{4*}, may designate a biradical consisting of 1-4 groups/atoms selected from $-C(R^aR^b)-$, $-C(R^a)=C(R^a)-$, $-C(R^a)=N-$, $-O-$, $-S-$, $-SO_2-$, $-N(R^a)-$, and $>C=Z$,

wherein Z is selected from -O-, -S-, and $-N(R^a)-$, and R^a and R^b each is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands,

said possible pair of non-geminal substituents thereby forming a monocyclic entity together with (i) the atoms to which said non-geminal substituents are bound and (ii) any intervening atoms; and

each of the substituents R^2, R^{2*}, R^3, R^{4*} which are present and not involved in the possible biradical is independently selected from hydrogen, optionally substituted C_{1-6}-alkyl, optionally substituted C_{2-6}-alkenyl, hydroxy, C_{1-6}-alkoxy, C_{2-6}-alkenyloxy, carboxy, C_{1-6}-alkoxycarbonyl, C_{1-6}-alkylcarbonyl, formyl, amino, mono- and di(C_{1-6}-alkyl)amino, carbamoyl, mono- and di(C_{1-6}-alkyl)-amino-carbonyl, amino-C_{1-6}-alkyl-aminocarbonyl, mono- and di(C_{1-6}-alkyl)amino-C_{1-6}-alkyl-aminocarbonyl, C_{1-6}-alkyl-carbonylamino, carbamido, C_{1-6}-alkanoyloxy, sulphono, C_{1-6}-alkylsulphonyloxy, nitro, azido, sulphanyl, C_{1-6}-alkylthio, halogen, photochemically active groups, thermochemically active groups, chelating groups, reporter groups, and ligands;

and basic salts and acid addition salts thereof;

with the proviso the monomer is not oxy-LNA.

Particularly preferred non-oxy-LNA monomers are 2'-deoxyribonucleotides, ribonucleotides, and analogues thereof that are modified at the 2'-position in the ribose, such as 2'-

O-methyl, 2′-fluoro, 2′-trifluoromethyl, 2′-O-(2-methoxyethyl), 2′-O-aminopropyl, 2′-O-dimethylamino-oxyethyl, 2′-O-fluoroethyl or 2′-O-propenyl, and analogues wherein the modification involves both the 2′and 3' position, preferably such analogues wherein the modifications links the 2'- and 3'-position in the ribose, such as those described by Wengel and coworkers (Nielsen et al., J. Chem. Soc., Perkin Trans. 1, 1997, 3423-33, and in WO 99/14226), and analogues wherein the modification involves both the 2′and 4' position, preferably such analogues wherein the modifications links the 2'- and 4'-position in the ribose, such as analogues having a -CH_2-S- or a -CH_2-NH- or a -CH_2-NMe- bridge (see Wengel and coworkers in Singh et al. J. Org. Chem. 1998, 6, 6078-9). Although, non-oxy-LNA monomers having the β-D-ribo configuration are often the most applicable, further interesting examples (and in fact also applicable) of non-oxy-LNA are the stereoisomeric of the natural β-D-ribo configuation. Particularly interesting are the α-L-ribo, the β-D-xylo and the α-L-xylo configurations (see Beier et al., Science, 1999, 283, 699 and Eschenmoser, Science, 1999, 284, 2118), in particular those having a 2'-4' -CH_2-S-, -CH_2-NH-, -CH_2-O- or -CH_2-NMe- bridge (see Wengel and coworkers in Rajwanshi et al., Chem. Commun., 1999, 1395 and Rajwanshi et al., Chem. Commun., 1999, submitted)

In the present context, the term "oligonucleotide" which is the same as "oligomer" which is the same as "oligo" means a successive chain of nucleoside monomers (*i.e.* glycosides of heterocyclic bases) connected via internucleoside linkages. The linkage between two successive monomers in the oligo consist of 2 to 4, preferably 3, groups/atoms selected from -CH_2-, -O-, -S-, -NR^H-, >C=O, >C=NR^H, >C=S, -Si(R")$_2$-, -SO-, -S(O)$_2$-, -P(O)$_2$-, -$PO(BH_3)$-, -P(O,S)-, -P(S)$_2$-, -PO(R")-, -$PO(OCH_3)$-, and -$PO(NHR^H)$-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl. Illustrative examples of such linkages are -CH_2-CH_2-CH_2-, -CH_2-CO-CH_2-, -CH_2-CHOH-CH_2-, -O-CH_2-O-, -O-CH_2-CH_2-, -O-CH_2-CH= (including R^5 when used as a linkage to a succeeding monomer), -CH_2-CH_2-O-, -NR^H-CH_2-CH_2-, -CH_2-CH_2-NR^H-, -CH_2-NR^H-CH_2-, -O-CH_2-CH_2-NR^H-, -NR^H-CO-O-, -NR^H-CO-NR^H-, -NR^H-CS-NR^H-, -NR^H-C(=NR^H)-NR^H-, -NR^H-CO-CH_2-NR^H-, -O-CO-O-, -O-CO-CH_2-O-, -O-CH_2-CO-O-, -CH_2-CO-NR^H-, -O-CO-NR^H-, -NR^H-CO-CH_2-, -O-CH_2-CO-NR^H-, -O-CH_2-CH_2-NR^H-, -CH=N-O-, -CH_2-NR^H-O-, -CH_2-O-N= (including R^5 when used as a linkage to a succeeding monomer), -CH_2-O-NR^H-, -CO-NR^H-CH_2-, -CH_2-NR^H-O-, -CH_2-NR^H-CO-, -O-NR^H-CH_2-, -O-NR^H-, -O-CH_2-S-, -S-CH_2-O-, -CH_2-CH_2-S-, -O-CH_2-CH_2-S-, -S-CH_2-CH= (including R^5 when used as a linkage to a succeeding monomer), -S-CH_2-CH_2-, -S-CH_2-CH_2-O-, -S-CH_2-CH_2-S-, -CH_2-S-CH_2-, -CH_2-SO-CH_2-, -CH_2-SO_2-CH_2-, -O-SO-O-, -O-S(O)$_2$-O-, -O-S(O)$_2$-CH_2-, -O-S(O)$_2$-

NR^H-, -NR^H-$S(O)_2$-CH_2-, -O-$S(O)_2$-CH_2-, -O-$P(O)_2$-O-, -O-P(O,S)-O-, -O-$P(S)_2$-O-, -S-$P(O)_2$-O-, -S-P(O,S)-O-, -S-$P(S)_2$-O-, -O-$P(O)_2$-S-, -O-P(O,S)-S-, -O-$P(S)_2$-S-, -S-$P(O)_2$-S-, -S-P(O,S)-S-, -S-$P(S)_2$-S-, -O-PO(R")-O-, -O-$PO(OCH_3)$-O-, -O-PO-(OCH_2CH_3)-O-, -O-$PO(OCH_2CH_2S$-R)-O-, -O-$PO(BH_3)$-O-, -O-$PO(NHR^N)$-O-, -O-$P(O)_2$-NR^H-, -NR^H-$P(O)_2$-O-, -O-$P(O,NR^H)$-O-, -CH_2-$P(O)_2$-O-, -O-$P(O)_2$-CH_2-, and -O-$Si(R")_2$-O-; among which -CH_2-CO-NR^H-, -CH_2-NR^H-O-, -S-CH_2-O-, -O-$P(O)_2$-O-, -O-P(O,S)-O-, -O-$P(S)_2$-O-, -NR^H-$P(O)_2$-O-, -O-$P(O,NR^H)$-O-, -O-PO(R")-O-, -O-$PO(CH_3)$-O-, and -O-$PO(NHR^N)$-O-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl, are especially preferred. Further illustrative examples are given in Mesmaeker et. al., Current Opinion in Structural Biology 1995, 5, 343-355 and Susan M. Freier and Karl-Heinz Altmann, Nucleic Acids Research, 1997, vol 25, pp 4429-4443. The left-hand side of the internucleoside linkage is bound to the 5-membered ring as substituent P^* at the 3'-position, whereas the right-hand side is bound to the 5'-position of a preceding monomer.

The term "succeeding monomer" relates to the neighbouring monomer in the 5'-terminal direction and the "preceding monomer" relates to the neighbouring monomer in the 3'-terminal direction.

Monomers are referred to as being "complementary" if they contain nucleobases that can form hydrogen bonds according to Watson-Crick base-pairing rules (e.g. G with C, A with T or A with U) or other hydrogen bonding motifs such as for example diaminopurine with T, inosine with C, pseudoisocytosine with G, etc.

When the modified oxy-LNA oligo contain at least two non-oxy-LNA monomers these may contain the same or different nucleobases at the 1'-position and be identical at all other positions or they may contain the same or different nucleobases at the 1'-position and be non-identical at at least one other position.

Accordingly, the present invention describes a method for degrading RNA *in-vivo* (in a cell or organism) or *in-vitro* by providing a high affinity oligonucleotide which activates RNaseH when the high affinity oligonucleotide is hybridised to a complementary RNA target sequence, said high affinity oligonucleotide may consist of oxy-LNA monomers exclusively.

Alternatively, the high affinity oligonucleotide may also consist of both oxy-LNA and non-oxy-LNA monomers, in this case the high affinity oligonucleotide contains at the most five, e.g. 4, e.g. 3 , e.g. 2 contiguous non-oxy-LNA monomers at any given position in the oligonucleotide, e.g. said high affinity oligonucleotide consists of both oxy-LNA and non-oxy-LNA monomers, wherein none of the non-oxy-LNA monomers are located adjacent to each other.

The high affinity oligonucleotide may also contain one or more segments of contigous non-oxy-LNA monomers. For instance, a stretch of contigous non-oxy-LNA monomers may be located in the centre of the oligonucleotide and with flanking segments consisting of oxy-LNA monomers. Alternatively the stretch of contigous non-oxy-LNA monomers may be located at either or both ends. Also, the oxy-LNA segement(s) may be either contigous or interrupted by 1 or more non-oxy-LNA monomers. Also, the high affinity oligonucleotide may comprise more than one type of internucleoside linkage such as for example mixes of phosphordiester and phosphorothioate linkages.

The resulting high affinity oligo containing oxy-LNA monomers and/or non-oxy-LNA monomers can thus be characterized by the general formula

$$5'\text{-}[X_mY_nX_p]_q\text{-}3'$$

X is oxy-LNA and Y is non-oxy-LNA, wherein m and p are integers from 0 to 30, n is an integer from 0 to 3 and q is an integer from 1 to 10 with the proviso that the sum of m+n+p multiplied with q is in the range of 6-100, such as 8, e.g. 9, e.g. 10, e.g. 11, e.g. 12, e.g. 13, e.g. 14, e.g. 15, e.g. 16, e.g. 17, e.g. 18, e.g. 19, e.g. 20, e.g. 21, e.g. 22, e.g. 23, e.g. 24, e.g. 25, e.g. 26, e.g. 27, e.g. 28, e.g. 29, e.g. 30, e.g. 35, e.g. 40, e.g. 45, e.g. 50, e.g. 60, e.g. 70, e.g. 80, e.g. 90, such as 100.

The present invention provides oligos which combine high affinity and specificity for their target RNA molecules with the ability to recruit RNAseH to an extend that makes them useful as antisense therapeutic agents. The oligos may be composed entirely of oxy-LNA monomers or they may be composed of both oxy and non-oxy-LNA monomers.

When both oxy-LNA and non-oxy-LNA monomer(s) are present in the oligo, the RNAseH recruiting characteristics of the chimeric oligo may be similar to, or different from, that of

the corresponding oxy-LNA oligo. Thus, in one aspect of the invention, non-oxy-LNA monomer(s) is/are used in such a way that they do not change the RNAseH recruting characteristics of the oxy-LNA/non-oxy-LNA chimeric oligo compared to the corresponding all oxy-LNA oligo. In another aspect of the invention the non-oxy-LNA monomer(s) is/are used purposely to change the RNAseH recruiting characteristics of an oxy-LNA oligo, either increasing or decreasing its efficiency to promote RNAseH cleavage when hybridised to its complementary RNA target compared to the corresponding all oxy-LNA oligo.

When both oxy-LNA and non-oxy-LNA monomer(s) is/are present in the oligo, the ability of the chimeric oligo to discriminate between its complementary target RNA and target RNAs containing one or more Watson-Crick mismatches may be different from the ability of the corresponding all oxy-LNA oligo to discriminate between its matched and mismatched target RNAs. For instance, the ability of an oxy-LNA oligo to discriminate between a complementary target RNA and a single base mismatched target RNA can be enhanced by incorporating non-oxy-LNA monomer(s), such as for instance DNA, RNA, thio-LNA or amino-LNA, either at, or close to, the mismatched position as described in applicant's Danish patent application entitled "Metod of increasing the specificity of oxy-LNA oligonuclotides" filed on the same day as the present application. Thus, in another aspect of the invention non-oxy-LNA monomer(s) is/are used purposely to construct an oxy-LNA/non-oxy-LNA oligo which exhibit increased specificity but unaltered RNAseH recruiting characteristics compared to the corresponding all oxy-LNA oligo. In another aspect of the invention the non-oxy-LNA monomer(s) is/are used purposely to construct an oxy-LNA/non-oxy-LNA oligo which exhibit both increased specificity and altered RNAseH recruiting characteristics compared to the corresponding all oxy-LNA oligo

Additionally, the oligonucleotide of the present invention may be conjugated with compounds selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens and peptides.

Examples

Example 1: LNA containing oligonucleotides recruit RNase H

Two 41-mer oligonucleotides, that make up a linearised double-stranded template for subsequent T7 polymerase run-off transcription, were used to obtain target RNA corresponding to the following 15mer oligonucleotides:

DNA control; 5'- gtgtccgagacgttg-3'
phosphorothioate control; 5'-*gtgtccgagacgttg*-3'
LNA gab-mer; 5'-GTGTccgagaCGTTG-3' (LNA in capital letters, DNA is small letters) and
LNA-mix-mer; 5'-gTgTCCgAgACgTTg-3' (LNA in capital letters, DNA is small letters)

In the 5'end of the sense template strand, the promoter sequence for T7 polymerase recognition and initiation of transcription were contained, followed by the DNA sequence coding for the target-RNA sequence. The two complementary oligonucleotides were heated to 80°C for 10min to produce the linearised double-strand template. A 20µl in vitro transcription reaction containing 500µM each of ATP, GTP and CTP, 12µM of UTP, approx. 50µCi of α-^{32}P UTP, 1 x transcription buffer (Tris-HCl, pH 7.5), 10mM dithiotretiol, 1% BSA, 20 U of RNasin ribonuclease inhibitor, 0.2 µl template and 250 units T7 RNA polymerase. The inclusion of RNasin inhibitor was to prevent degradation of the target-RNA from ribonucleases. The reactions were carried out at 37°C for 2h to produce the desired 24mer ^{32}U-labelled RNA run-off transcript. For target RNA purification, 1.5µl (1.5 Units) of DNase I was added to the RNA which was resolved in a 15% polyacrylamide gel containing 7M urea and the correctly sized fragment was excised from the gel, dispensed in elution buffer (0.1% SDS, 0.5M ammonium acetate, 10mM Mg-acatate) and incubated at room temperature overnight. The target RNA sequence was then purified via ethanol precipitation, the supernatants filtered through a Millipore (0.45m) and collected by ethanol precipitation. The pellets were diluted in TE-buffer and subsequently subjected to RNAse H digestion assay. Herein, the decrease of intact substrate, *i.e.* the 24-mer α-^{32}P UTP labelled target RNA sequence, was assayed over time as follows. The reactions were carried out in a total volume of 110µl and contained (added in the order mentioned): 1 x nuclease-free buffer (20mM Tris-HCl, pH 7.5, 40mM KCl, 8mM $MgCl_2$, 0.03 mg/ml BSA), 10mM dithiotretiol, 4% glycerol, 100nM of oligonucleotide, 3 Units RNasin inhibitor, labelled target RNA strand and 0.1 U of RNase H. An excess of oligonucleotide was added to each reaction to ensure full hybridisation of the RNA target sequences. Two

negative controls were also included and were prepared as above but (1) without any oligonucleotide, or (2) without RNase H added to the reaction mixture. All the reactions were incubated at 37°C. At time points 0, 10, 20, 40 and 60 min., 10µl aliquots were taken and immediately added to ice-cold formamide loading buffer to quench the reaction and stored at –20°C. The samples were heated to 85°C for 5 min. prior to loading and running on a 15% polyacrylamide gel containing 7M urea. The gels were vacuum dried and exposed to autoradiographic films over night and subsequently subjected to densitometric calculations using the Easy Win imaging software (Hero Labs). The volume density of intact target RNA were calculated in each lane with correction for background. The volume density for the time zero sample was set as reference value for each incubation. Relative values for the other time-points samples in the corresponding incubation were calculated based on these reference values.

Brief description of figures

Figure 1 shows the results of the RNase H experiments. As expected the control DNA and phosphorothioate oligonucleotides both recruit RNAse H very efficiently. Also, as expected the LNA oligonucleotide which contains a contiguous stretch of six DNA monomers in the middle (LNA gab-mer) recruits RNAse H efficiently. Surprisingly, however, the LNA mix-mer which contains only single DNA monomers interdispersed between LNA monomers also recruits RNAse H. We conclude that the activity of RNase H is not contingent on a contiguous stretch of DNA or phosphorothioated bases when LNA is used as a component of the oligonucleotide.

Claims

1. A method for degrading RNA comprising providing a high affinity oligonucleotide which recruits RNaseH when hybridised to an RNA target sequence, wherein said high affinity oligonucleotide consists of oxy-LNA monomers exclusively.

2. A method for degrading RNA comprising providing a high affinity oligonucleotide which recruits RNaseH when hybridised to an RNA target sequence, wherein said high affinity oligonucleotide consists of both oxy-LNA and non-oxy-LNA monomers and wherein there are at the most five contiguous non-oxy-LNA monomers at any given position in the oligonucleotide.

3. A method for degrading RNA comprising providing a high affinity oligonucleotide which recruits RNaseH when hybridised to an RNA target sequence, wherein said high affinity oligonucleotide consists of both oxy-LNA and non-oxy-LNA monomers and wherein none of the non-oxy-LNA monomers are located adjacent to each other.

4. A method according to any of claims 2 or 3, wherein the presence of the non-oxy-LNA monomer(s) in the oxy-LNA/non-oxy-LNA oligo does not change the RNAseH recruiting characteristics of the oligo compared to the corresponding oxy-LNA oligo.

5. A method according to claim 4, wherein the presence of the non-oxy-LNA monomer(s) in the oxy-LNA/non-oxy-LNA oligo modifies the RNAseH recruiting characteristics of the oligo compared to the corresponding oxy-LNA oligo.

6. A method according to claim 5, wherein the presence of the non-oxy-LNA monomer(s) in the oxy-LNA/non-oxy-LNA oligo either enhances or reduces the ability of the oligo to recruit RNAseH compared to the corresponding oxy-LNA oligo.

7. A method according to any of the claims 2 to 6 wherein the presence of the non-oxy-LNA monomer(s) in the oxy-LNA/non-oxy-LNA oligo increases the ability of the oligo to discriminate between its complementary target RNA and target RNAs containing one or more Watson-Crick mismatches compared to the corresponding oxy-LNA oligo.

8. A method according to any of the previous claims wherein the oligonucleotide is characterised by the general formula

$$5'\text{-}[X_mY_nX_p]_q\text{-}3'$$

wherein X is oxy-LNA and Y is non-oxy-LNA, wherein m and p are integers from 0 to 30, n is an integer from 0 to 5 and q is an integer from 1 to 10.

9. A method according to claim 2-8, wherein the non-oxy-LNA monomer(s) is/are deoxyribonucleotide(s).

10. A method according to claim 9, wherein the deoxyribonucleotide is modified at the 2'-position in the ribose.

11. A method according to claim 10, wherein the 2'-modification is a hydroxyl, 2´-O-methyl, 2´-fluoro, 2´-trifluoromethyl, 2´-O-(2-methoxyethyl), 2´-O-aminopropyl, 2´-O-dimethylamino-oxyethyl, 2´-O-fluoroethyl or 2´-O-propenyl.

12. A method according to claim 11, wherein the modification also involves the 3' position, preferably modifications that links the 2'- and 3'-position in the ribose.

13. A method according to claim 12, wherein the modification also involves the 4' position, preferably modifications that links the 2'- and 4'-position in the ribose.

14. A method according to claim 13, wherein the modification is selected from the group consisting of a 2'-4' link being a $-CH_2-S-$, $-CH_2-NH-$, or $-CH_2-NMe-$ bridge.

15. A method according to any of the claims 9 to 14, wherein the nucleotide has the α-D-ribo, β-D-xylo, or α-L-xylo configuration.

16. A method according to any of the claims 9 to 15, wherein either all or some of the oxy-LNA monomers or all or some of the non-oxy-LNA monomer(s) or all or some of both the oxy-LNA monomers and non-oxy-LNA monomer(s) contain a 3´- or 5´- modification that results in an internucleoside linkage other than the natural phosphorodiester linkage.

17. A method according to claim 16, wherein the modification is selected from the group consisting of -O-P(O)$_2$-O-, -O-P(O,S)-O-, -O-P(S)$_2$-O-, -NR^H-P(O)$_2$-O-, -O-P(O,NR^H)-O-, -O-PO(R")-O-, -O-PO(CH$_3$)-O-, and -O-PO(NHR^N)-O-, where R^H is selected form hydrogen and C_{1-4}-alkyl, and R" is selected from C_{1-6}-alkyl and phenyl.

18. A method according to any of the preceding claims, wherein the incorporation of the at least one non-oxy-LNA monomer changes the affinity of the resulting oligo towards its complementary nucleic acid compared to the affinity of the all-oxy-LNA oligo by a ΔT_m of no more than ± 5°C.

19. A method according to claim 18, wherein the affinity is changed by no more than ± 10°C.

20. A method according to any of claims 18 or 19, wherein at least two non-oxy-LNA monomers containing either the same or different nucleobases at the 1'-position and being identical at all other positions are used.

21. A method according to any of claims 18 or 19, wherein at least two non-oxy-LNA monomers containing either the same or different nucleobases at the 1'-position and being non-identical in at least one other position are used.

22. A oligomer according to any of the previous claims, wherein said oligomer is used as a therapeutic compound, e.g. as an antisense compound.

23. An oligomer as defined in any of the previous claims, which is conjugated with compounds selected from proteins, amplicons, enzymes, polysaccharides, antibodies, haptens, and peptides.

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Figure 1.